PREVALENCE OF ULCERATIVE DISEASE IN FREE-RANGING KREFFT'S TURTLE

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Clinical evidence is documented for an ulcerative skin disease of unknown cause which is prevalent in a population of Krefft's turtles (*Emydura krefftii*) in east-central Queensland. The population in Callide Dam near Biloela was the sole known focus of disease, despite intensive surveys of surrounding catchments. Fresh or regressing skin lesions occurred in 39% of 869 turtles caught over 7 sampling occasions (range 25-84%). We document disease prevalence within the population and preliminary histopathological findings. Similar infectious diseases in other aquatic vertebrates are reviewed. ☐ *Uulcer, disease, turtle, Emydura krefftii.*

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There is an increasing trend in numbers of reports of disease in chelonians; emerging infectious diseases are now known for all the major groups, including sea turtles (viral fibropapillomas), freshwater turtles (ulcerative shell necrosis) and land tortoises (upper respiratory tract infections) (Herbst, 1994; Lovich et al., 1996; Garner et al., 1997; Berry, 1997; Ernst et al., 1999). To reiterate Jacobsen (1997), it is imperative to distinguish whether recently documented diseases reflect an onset of degraded environmental conditions (Cann, 1993), biomagnification of chemical toxicants in the environment (Ernst, 1997) or an increase of interest in these animals.

There is limited awareness about the health status of Australian chelids, despite a burgeoning concern for the status of turtle populations elsewhere (Ernst, 1997). Cann (1993,1998) drew attention to the *Elseya bellei* populations of the Namoi River, NSW which suffer from blindness, and also an *Emydura* population in Victoria which declined at a time of toxic algal blooms (Cribb, 1991). Apart from these qualitative observations, we are not aware of any quantitative accounts of disease in free-ranging freshwater turtles in Australia. Nevertheless, reports are received sporadically about localised die-offs of freshwater turtles in SE Queensland. Since 1995, mass mortalities within isolated small farm impoundments were noted with *Elseya latisternum* and *E. signata* in the Brisbane and Pine Rivers catchments and with *Chelodina longicollis* in the upper Darling Downs Catchment as well as the upper Dawson River (C. Limpus unpublished data). It is unknown if disease, contamination or natural environmental variation is implicated since none of the die-offs occurred in conjunction with fish kills. Timely documentation and follow-up are required if forensics are to be useful in such episodes.

This paper documents clinical, epidemiological and pathological features of a skin disease of unknown cause which became prevalent in a single population of Krefft's turtles (*Emydura krefftii*) in east-central Queensland. This population is the sole known focus of disease at present, despite intensive surveys of the surrounding catchments.

METHODS

Surveys of the Fitzroy, Kolan, Burnett and Mary River were initiated by the Queensland Parks and Wildlife Service in 1997-1999 to assess the effects of dams and weirs on freshwater turtle populations (Tucker, 2000). The only population that displayed evidence of disease (among >10,000 turtles collected from 28 riverine sites and 26 dam sites) was at Callide Dam (24°21.793S, 150°38.650E; 10km E of Biloela). Following the first recognition of the disease in July 1998, we conducted follow-up surveys at irregular intervals over the next year to monitor temporal variation in prevalence of clinical signs (survey dates are given in Table 1).

TABLE 1. Summary of Krefft's turtles that displayed symptoms of active or healing ulcerations when encountered during surveys of Callide Dam. Note: total turtles sampled includes 5 recaptured turtles, of which 3 had new ulcers when recaptured.

Date of sampling	Ulcers or recent scars present	Total turtles sampled	% diseased
11/7/98	72	220	32.7
30/8/98	42	70	58.3
15/12/98	21	25	84.0
17/2/99	65	242	26.9
25/2/99	88	180	48.9
15/4/99	49	116	42.2
27/5/99	4	16	25.0
Overall	341	869	39.2

STUDY SITE. Callide Dam impounds Callide Creek to provide cooling water to an adjacent coal-fired power generating plant situated immediately to the northwest and for irrigation of an intensive cotton-growing industry of the surrounding region. To the north is one of the Callide coalfields that extract low-grade sulphur coal for the power plant. The large cooling towers are monitored to strict environmental standards so that any water taken from Callide Dam for cooling or furnace ash washing is self-contained and recirculated within settling ponds on the plant grounds. Some surface runoff from the plant and mine may enter the lake. However, the role of groundwater transport or agricultural runoff is not monitored.

Water quality of Callide Dam is tested by a local Queensland Dept of Natural Resources laboratory in Biloela (Allen, 1982). The waters that feed Callide are pumped uphill from Awoonga Dam, a coastal catchment separated by a low mountain range. The water pH is 7.7-7.9 as it enters Callide Creek. The lake stratifies and has documented high levels of nutrients. Fisheries surveys in Callide Dam have recorded pH of 8.0-8.5 consistently over the past decade (P. Long-QDPI, unpubl. data).

STUDY ANIMALS. Krefft's turtle (*E. krefftii*) is a common chelid turtle in rivers, reservoirs and farm ponds of SE Queensland (Cann, 1998). The Fitzroy region hosts a high biodiversity of turtle species (*E. krefftii*, *Rheodytes leukops*, *E. latisternum*, *Elseya* sp., *Chelodina expansa*, *C. longicollis* and *C. novaeguinea*, with a hybrid zone between the latter two). Because *E. kreffiii* is the least specialised of these turtle species in its

habitat and diet, it predominates in permanent aquatic habitats (Tucker, 2000).

The *E. krefftii* in Callide Dam are distributed at high density in the shallower margins of the lake, where waters are regularly anoxic and thermally stratified (QPWS, unpubl. data). These conditions may be exacerbated because Callide Dam has remained at low storage levels for many years (QDNR water storage reports).

SPECIMEN PROCESSING. Turtles were obtained on two initial occasions by seining and thereafter by baited crab traps. Turtles were kept shaded in mesh bags or bins during processing. All turtles were returned to the vicinity of capture within 24 hrs, except for specimens that died during processing or that were retained for necropsy. We took standard measurements of length (carapace and plastron length were measured to the nearest 1mm with callipers) and weight (measured to the nearest 1g on an electronic balance) of each turtle. A body condition index was assessed from the residuals for a regression of carapace length to mass by the equation mass $= a*(SVL)^b$.

In the Callide population, adult turtles (carapaces > 20cm) were reliably sexed by dimorphism of the tail. Males have a thickened elongate tail with the vent position posterior to the rear margin of the carapace; females have a relatively short tapered tail with the vent not extending beyond the rear margin of the carapace. For turtles < 20cm that displayed no dimorphism, we viewed the gonads directly via laparoscopy to determine sex (detailed methods given in Limpus et al., 2002). Each turtle was individually marked by a numbered metal tag inserted in the webbing of the rear foot. Each turtle was inspected visually for ulcers on the skin of the neck and shoulder region. Ulcers were coded as 0, 1, or 2 respectively for no evidence, active ulceration, or remnant scar tissue from what was assumed to have been a previous ulcer. Recaptures of tagged individuals that had been affected previously were followed to record cases of ulcer progression or recovery.

On 17 February 1999, 6.2% of the sampled animals (n=15/242) died while being held for processing. Deaths were from 4.5% (4/88 of males, 7.7% (11/143) of females, and no juveniles. Such mortality had not been recorded elsewhere and there had been no significant change in our processing procedures, which had previously resulted in negligible mortality. We viewed the mortality with apprehension because

of the external ulcers and what we subjectively assessed as poor condition in the population. The 15 carcasses were frozen for later examination. Detailed pathological examination was not performed on these specimens due to the freeze-thaw artifact.

We returned on 25 February to collect a sample of live turtles with both symptomatic and normal appearance. Specimens were transported to the Veterinary Pathology lab at University of Queensland where they were submitted for clinical, necropsy and histopathological examination on 26 February.

Complete necropsy dissection was performed on one normal and 4 turtles with skin ulcers, immediately after euthanasia by intravenous injection of pentobarbitone sodium solution. Haematoxylin and eosin-stained sections of formalin-fixed skin and liver tissue were cut from paraffin-embedded blocks.

Dietary samples were obtained by stomach-flushing 10 turtles on 15 December 1998. Stomach samples were preserved in 70% ethanol and identified later with a dissecting microscope and field guides. Prey species were quantified by percent occurrence.

RESULTS

Fresh or healed skin ulcers occurred in 39% of 869 turtles caught over 7 sampling occasions (range 25-84%) (Table 1). Affected turtles had fresh or healing ulcers on the dorsal aspect of the neck or shoulder region. Lesions were equally common in males, females, and juveniles. Recaptures of individual turtles were sparse (n= 5), but of these, 2 turtles remained unaffected, and 3 turtles had become ulcerated between captures. Recapture numbers were insufficient to assess the outcome of the disease (healing or progression), given that recapture was dependant on survival as well as the probability of recapture. An alternative was to contrast the percentages of turtles with active and regressive ulcers (39%, Table 1) to those which only had active ulcers (18%, Table 2). A 21% difference was a representative index of turtles with ulcers that would be expected to regress.

Analysis of variance indicated that affected males, females, and juveniles had a significantly lower length (F = 71, P = 0.0001), mass (F = 141, P < 0.001), and mass/length ratio (F = 109, p < 0.0001) than unaffected individuals (Table 2). Residuals from the power function relating length to body mass (mass = $a*(SVL)^b$; a=

TABLE 2. Comparisons of length, mass, and condition for 'normal' and affected *Emydura krefftii* at Callide Dam. Table values are means with 1 s.e. in parenthesis. Sex is abbreviated as F-female, M-male, I-immature with a − or + sign indicating absence (82%) or presence (18%) of lesions, respectively. Note: mass data were unavailable on 20 turtles (for logistic reasons), so sample sizes differ from Table 1. Negative residuals in the right column indicated animals in poorer body conditions than expected for the population, and were calculated as a body condition index from regressions of body length to mass given in Fig. 1.

Turtle	N	Length (cm)	Mass (kg)	Ratio kg/cm	Body Condition (residual)
F-	416	24.05 (0.14)	1.707 (0.027)	0.069 (0.001)	0.249 (0.055)
F+	80	23.15 (0.39)	1.533 (0.068)	0.063 (0.002)	-0.062 (0.126)
M-	237	21.69 (0.19)	1.173 (0.027)	0.052 (0.001)	-0.334 (0.049)
M+	63	20.85 (0.40)	1.043 (0.055)	0.048 (0.001)	-0.325 (0.081)
I-	43	12.58 (0.49)	0.272 (0.029)	0.019 (0.001)	0.002 (0.029)
I+	10	12.72 (0.48)	0.239 (0.021)	0.018 (0.001)	-0.053 (0.044)

0.000088775, s.e. = 0.0000101866 ; b=3.08146, s.e.=0.035723) indicated whether an individual was greater than or less than the population standard for animals of given size; for the Callide turtles, these responses were unique by sex (Table 2). Unaffected females had positive residuals relative to affected females, both groups of males had negative residuals of similar magnitude, while both groups of juveniles displayed residuals not significantly different from the baseline. In other words, only unaffected females weighed more than expected for their respective size. Plots of ln(SVL) against ln(mass) indicated that the residuals were not a result of different slopes or intercepts by sex, as all plots coincided on a single regression line. However, without knowing the contributions of muscle, oedema fluid and fat to body weight of these live turtles, we cannot say whether a heavier than average turtle for its size (noted by a positive residual) resulted from fairly extensive fat deposits or some pathology such as oedema or ascites, etc.

The ten stomach samples revealed that *E. krefftii* foraged across multiple trophic levels in the reservoir. Six turtles consumed submerged aquatic plants (*Myriophyllum* sp., *Ceratophyllum* sp.), 5 ate inundated terrestrial grasses, 4 consumed bivalves, 3 scavenged fish, 3 ingested
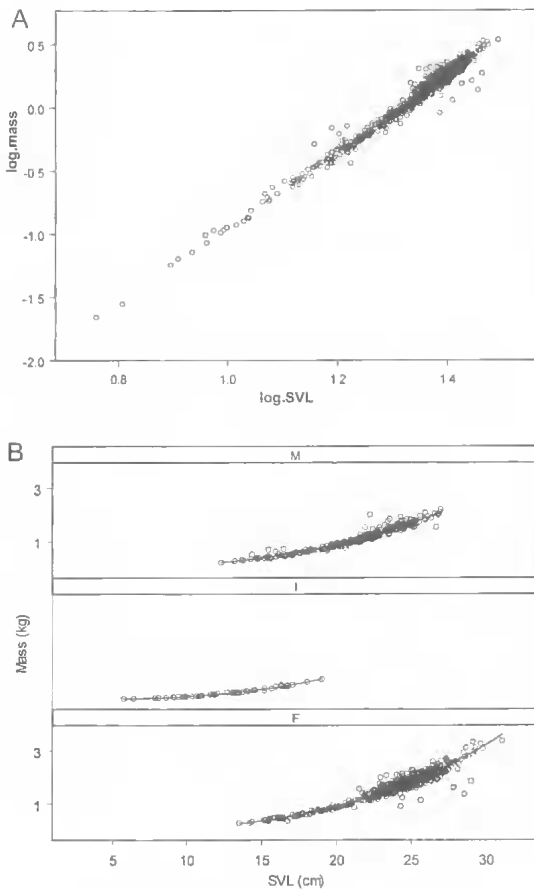
FIG. 1. A, coincidence of all points to a common regression line, regardless of sex. B, points conditioned by sex to indicate relationship of length to body mass (mass = $a*(SVL)^b$; a = 0.000088775, s.e. = 0.0000101866 ; b = 3.08146, s.e. = 0.035723) for Krefft's turtles (*E. krefftii*) in Callide Dam.

gum leaves as windfall, and 2 had consumed filamentous algae. Although aquatic plants are often ingested incidentally as turtles consume aquatic insect larvae, there were no traces of larvae found in the samples.

Clinical examination of 3 males and 1 female revealed single small sunken, puckered circular scars ranging from 3-5mm diameter on the dorsal neck skin just behind the head of each animal. All animals appeared vigorous. Necropsy revealed minimal scarring of the soft tissues beneath the skin lesions. All animals had adequate internal fat reserves and there were no gross abnormalities apparent at necropsy.

Histological examination confirmed that the skin lesions were ulcers, covered by a dense diphtheritic membrane, overlying a fairly narrow zone of low-grade sub-acute inflammation. These ulcers were at more or less the same stage of resolution and did not appear to show a progression toward deeper infection. The cause of these ulcers was not apparent: there was no evidence in these sections of superficial or deep fungal invasion. The only other tissue examined histologically was liver, which showed marked fat accumulation in hepatocytes, and was judged to be normal, since the liver from the non-ulcerated animal was similar to those of the ulcerated turtles.

DISCUSSION

In summary, a previously unrecognised focal ulcerative skin disease has been recognised as occurring in *E. krefftii* in a single water impoundment over a two-year period. At the beginning of this period there was an unexplained mortality of 6% in a batch of recently-captured *E. krefftii*, but the cause of this mortality was not determined, nor was it established that there was any connection between the ulcerative disease and the mortality. Disease expression was variable over the year, but the irregular sampling protocol was insufficient to detect any seasonality in the condition (this investigation was during a regional survey into the effects of river regulation on freshwater turtle ecology (Tucker, 2000)).

The skin lesions of affected *E. krefftii* were only superficially similar to septicemic cutaneous ulcerative disease, which in aquatic turtles is characterised by cutaneous ulceration, anorexia, lethargy, and late in the course of the disease, hepatic necrosis, paralysis, and death (Frye, 1981). The infection is associated with the bacterium *Citrobacter freundii* and soft-shelled turtles (Trionychidae) are the group most frequently reported with the infection (Kaplan, 1957). However, such bacterial infections are most often secondarily associated with dermal abrasion of the plastron during handling of wild turtles or abrasions on captive turtles (Frye, 1981). The history and location of ulcers on the wild *E. krefftii* was inconsistent with abrasions due to capture. Several fungi are associated with lesions of the integument in aquatic chelonians, but keratin of the carapace is affected (Lovich et al., 1996; Garner et al., 1997) rather than dermis, as for example, *Basidiobolus ranarum* (Nickerson & Hutchinson, 1971). Another aquatic disease with clinical signs of ulcerative

myeosis is the pathogenic fungus *Mucor amphibiorum* (Munday et al., 1998), although this has not been reported for turtles. However, no fungal hyphae were noted in the histological sections so thus far fungal infection has not been implicated with the disease at Callide Dam.

Apart from bacterial and fungal agents, viral diseases pose threats to wildlife health. A recent review of emerging infectious disease in animals notes that ranaviruses, herpesviruses, and iridoviruses are common in aquatic organisms (Daszek et al., 1999).

Because the waters of Callide Dam are not used as a potable water source, limited data are collected on water conditions beyond biological oxygen demand or cyano-bacteria counts. Basic water pH (Allen, 1982) and lack of elevated sulphates or sulphites would seem to preclude links to acidic runoff conditions. That might rule out several fish diseases (such as red spot, when cutaneous ulcers are evident) that develop seasonally. Aromatic hydrocarbons, another potent skin irritant, are not monitored nor is there any systematic screening for pesticide compounds from local agricultural runoff (DNR Water Resources, pers. comm.).

Effective turtle conservation includes a recognition and proactive monitoring of threats (Klemens, 2000). Little is known about the immune systems of reptiles in general (Cooper et al., 1985) and the present knowledge of aquatic turtle diseases is derived principally from captive turtles as opposed to wild populations. Thus further study of Callide turtles may provide insight on disease processes in other free-ranging turtles. Conditions of high population density, low storage levels that create food-depleted conditions, highly stratified, anoxic water quality, and elevated levels of cyanobacteria or eutrophic conditions may be conducive to disease expression and transmission in aquatic turtles.

The health status of turtle populations is a vital concern because their life histories are poorly buffered against incidental mortality of later life history stages (Heppell, 1998), as in this case for adult females. Further investigation is needed to determine whether a disease of Callide *E. krefftii* might be transmitted to other aquatic species of the Fitzroy catchment. As the endemic Fitzroy Turtle (*Rheodytes leukops*) and an undescribed species of *Elseya* sp. are of specific conservation concern in the Fitzroy Catchment, measures may be warranted to contain a presently uncharacterised disease. If an infection cause is established

for the condition, precautions may include preventive quarantine of Callide turtles (whether taken during fishing contests or for pets) and disinfection of equipment after boating or trapping in the area. Turtles might also serve as active vectors since turtle movements during droughts are directed toward deeper reaches of river or overland to remnant water bodies (Cann, 1998). Furthermore, turtles may be flushed downstream during reservoir overflows. Such conditions have potential to readily disperse diseased individuals beyond the confines of Callide Dam.

Future studies are confronted by two major challenges: to determine the aetiology and pathogenesis of a new disease and whether it poses a disease risk to other aquatic species. A first step is to study turtles at earlier stages of the disease. Such investigations should include attempts to isolate pathogens from acutely affected individuals, followed by transmission trials of candidate isolates. Further work should be directed at determining the outcome (death, breeding compromise or recovery, etc) of the uncharacterised disease.

ACKNOWLEDGEMENTS

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